7. X-RAY EFFECT ON THE ULTRASTRUCTURE OF THE POLLEN GRAINS OF PINUS GRIFFITHII McCLELL.

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Abstract

The transmission electronmicroscopical results of X-ray irradiated pollen grains of *Pinus griffithii* McCLELL pollen grains are presented in this paper. Irradiation with CuKα X-ray, at 35 KV, 20 mA, during 5', 15' 35' and 60'. The sporopollenin biopolymer system is resistant to irradiation similarly to that of *Ginkgo biloba* L. Subunits were not observed in contrast to the partial dissolutions method. But after 60' of irradiation molecular structures (sensu strictu) were observed in particular at the endexine/intine border. Microbial organisms were observed not only on the surface of the pollen grains, but within the ectexine, in the cavities of the infratectal layer, mostly in the alveolar system of the bladders.

Key words: Palynology, recent, *Pinus griffithii*, X-ray effect, TEM.

Introduction

Pollen grains of *Pinus griffithii* McCLELL are very important in one of the research programs of our Laboratory. The quasi-crystalloid basic biopolymer skeleton was first discovered in the partially degraded pollen grains of this species (KEDVES 1988a,b). Later (KEDVES, 1989a) the first method of investigation of the biopolymer symmetry of the metastable skeleton was published, and in another paper (KEDVES 1989b) three degrees have been distinguished at the highly organized biopolymer units of the sporoderm. Symmetry operations of the biopolymer structure in angstroem dimension were published in several papers on the partially degraded pollen grains of *Pinus griffithii* McCLELL (KEDVES, KINCSEK, AMBRUS, FEJES and GYEBROVSZKI 1990, KEDVES 1990, 1991, KEDVES, PÁRDUTZ, FARKAS and VÉR 1991, KEDVES, TÓTH, FARKAS, BELLON and SCHMÉL 1992, KEDVES, TÓTH and GOTTL, 1994). In 1993, KEDVES, TÓTH and FARKAS published the first rotation results in a pentagonal molecule of *Pinus griffithii* exine. The symmetry operations verified that the quasi-crystalloid skeleton or lattice is present in the biopolymer system on molecular level, too. To the stabilizing biopolymer system of the metastable quasi-crystalloid skeleton the first TEM data were published by KEDVES and TÓTH (1994). Further molecular structures of the partially dissolved foot layer and endexine were described by KEDVES, TÓTH, KÁROSSY and VARGA (1996).

Further data concerning the experimental results on *Pinus griffithii* pollen grains were published by KEDVES and PÁRDUTZ (1992) and KEDVES and GÁSPÁR (1994).

Till this time we have TEM data of the X-ray irradiated pollen grains of the following species: *Alnus glutinosa* (L.) GAERTN. (KEDVES and PÁRDUTZ, 1992), *Ginkgo biloba* L.

(KEDVES and PÁRDUTZ, 1997), *Ustilago maydis* (DE CANDOLLE) CORDA (KEDVES, PÁRDUTZ and BORBOLA, 1997).

LM data of the X-ray irradiated *Pinus griffithii* pollen grains were published by KEDVES and GÁSPÁR (1995a,b).

The aim of this paper to get comparative TEM data of the X-ray irradiated *Pinus griffithii* pollen grains. Our paper (KEDVES, HEGEDÜS and OLÁH, 1992) was used to the terminology of the LM morphology of the bisaccate *gymnosperm* pollen grains.

Materials and Methods

The investigation material was collected in the Botanical Garden of the J. A. University 16.05.1990, by I. OLÁH and A. HEGEDÜS. After collection the pollen grains were frozen at –20 °C to diminish the alterations of the biopolymer system. The irradiations were made 05.08.1993, with a BRON-OM1 apparatus in the Radiological Laboratory of the Department of Mineralogy, Petrology and Geochemistry of the J. A. University, Szeged. Radiaton data: 35 KV, 20 mA, CuKα beam. Length of time and numbers of experiments: 5' 1736, 15' 1737, 35' 1738, 60' 1739. After irradiation the pollen material was postfixed with 1.0% OsO_4 aquous dilution and embedded in Araldite. The ultrathin sections were made at the Hungarian Academy of Sciences Biological Research Center EM Laboratory on a Porter Blum ultramicrotome. The TEM photographs were taken on an Opton EM-902 (resolution 2–3 Å), and on a Tesla BS-540 (resolution 5 Å).

Results

The ultrastructure of the non-experimental fresh pollen grains was described previously (KEDVES 1989, planche 1, 1,2). Beneath the foot layer it is a thin endexine which seems to be non-structured.

Experiment No: 1736 (Plate 7.1., figs. 1,2). – Alterations were not observed in the fine structure of the outer part of the bladders (Plate 7.1., fig. 1). The ultrastructure of the exine of the corpus was investigated on semi-tangential sections (Plate 7.1., fig. 2). Similarly to the bladders the substance of the exine was not altered.

Experiment No: 1737 (Plate 7.1., figs. 3–5). The fine structure of the exine of the corpus is not altered. The ultrastructure of the foot layer and the endexine is very similar to that of the non-experimental pollen grains. No alterations were observed in the fine structure of the bladders (Plate 7.1., fig. 4). At the proximity of the corpus/bladder border, the foot layer separated from the endexine, and the endexine is lamellar.

Experiment No: 1738 (Plate 7.2., figs. 1–4). – No important alterations were observed in the ultrastructure of the exine of the corpus (Plate 7.2., figs. 1,2). The endexine is separated from the foot layer, which is more or less homogeneous. Sometimes the electron density of the endexine is strong (Plate 7.2., fig. 2). The ultrastructure of the endexine at the corpus/saccus border is also homogeneous, the ectexine is without alterations. Within the holes of the alveolar system of the bladders microbial organisms were observed (Plate 7.2., fig. 3).

Experiment No: 1739 (Plate 7.3., figs. 1,2, plate 7.4., 7.5.). – The exine surface in the germinal area (Plate 7.3., figs. 1, 2) is full of microorganisms. The ectexine, including the foot layer disappears in the apertural area. The endexine is homogeneous, its electron density is strong. In the highly magnified pictures of the ectexine (Plate 7.4., figs. 1,2,

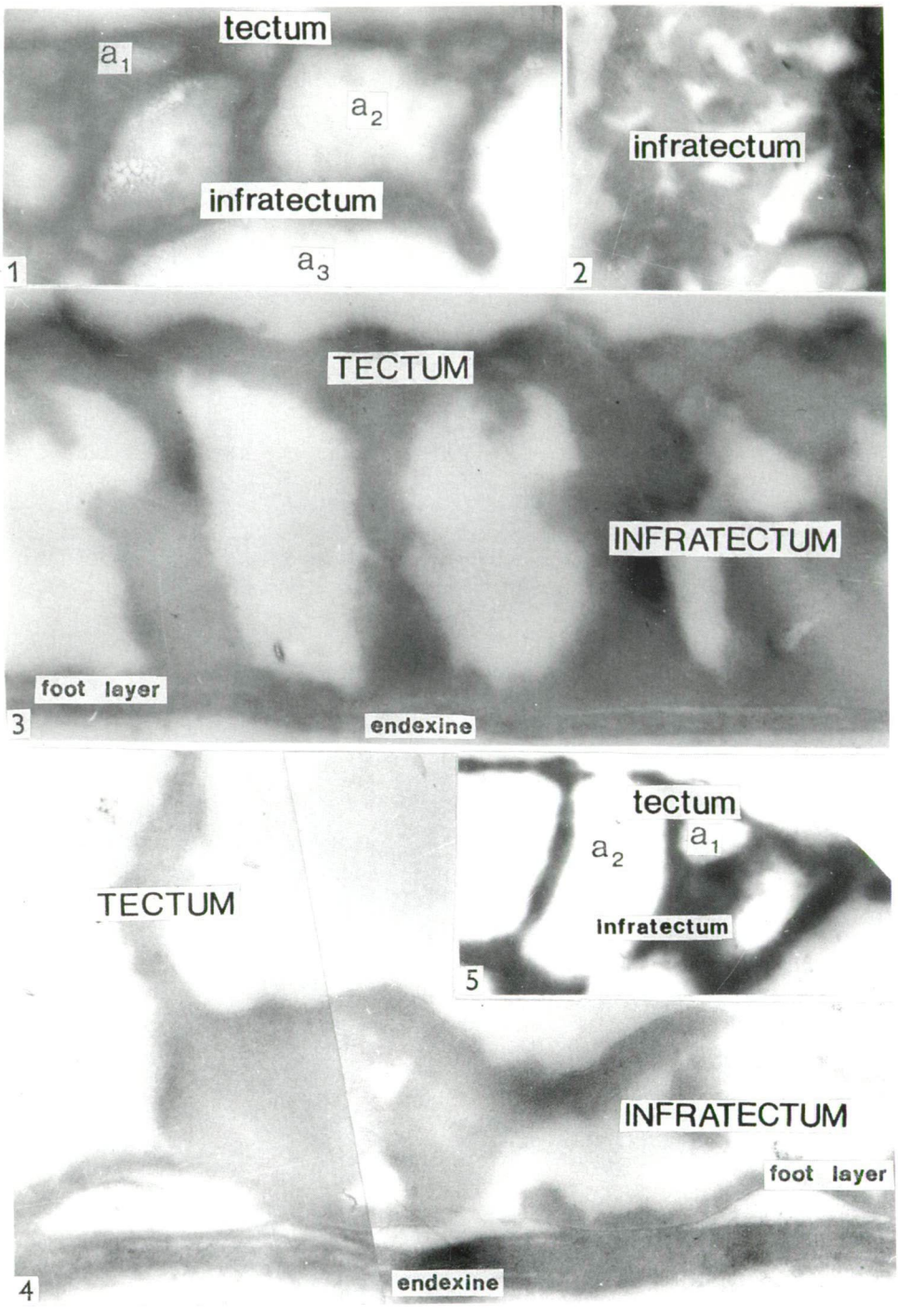
tectum
a1
a2
infratectum
a3
1
infratectum
2
TECTUM
INFRATECTUM
foot layer
endexine
3
TECTUM
tectum
a2
a1
infratectum
5
INFRATECTUM
foot layer
endexine
4

Plate 7.1.

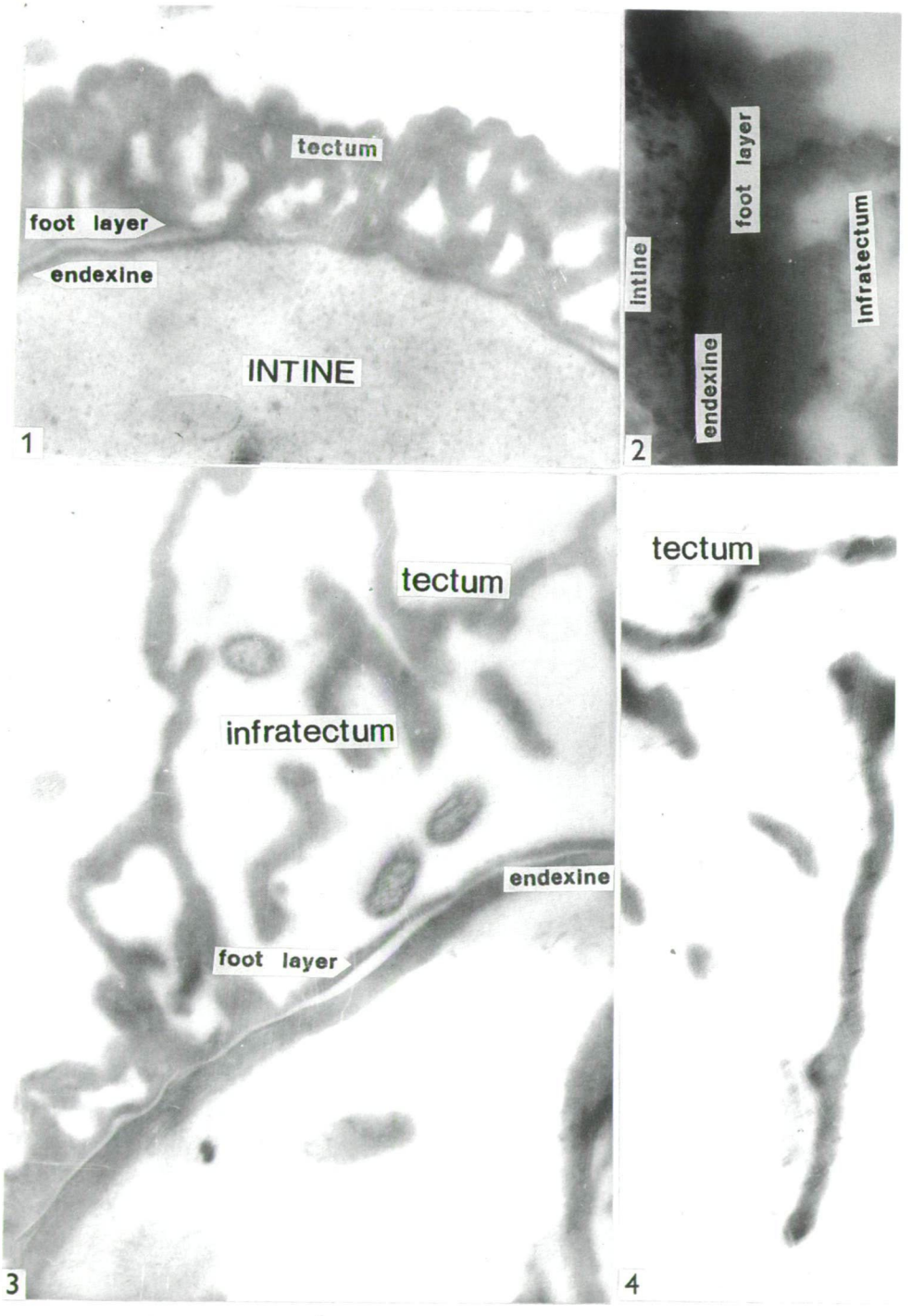
tectum
foot layer
endexine
INTINE
1
intine
foot layer
infratectum
endexine
2
tectum
infratectum
endexine
foot layer
3
tectum
4

Plate 7.2.

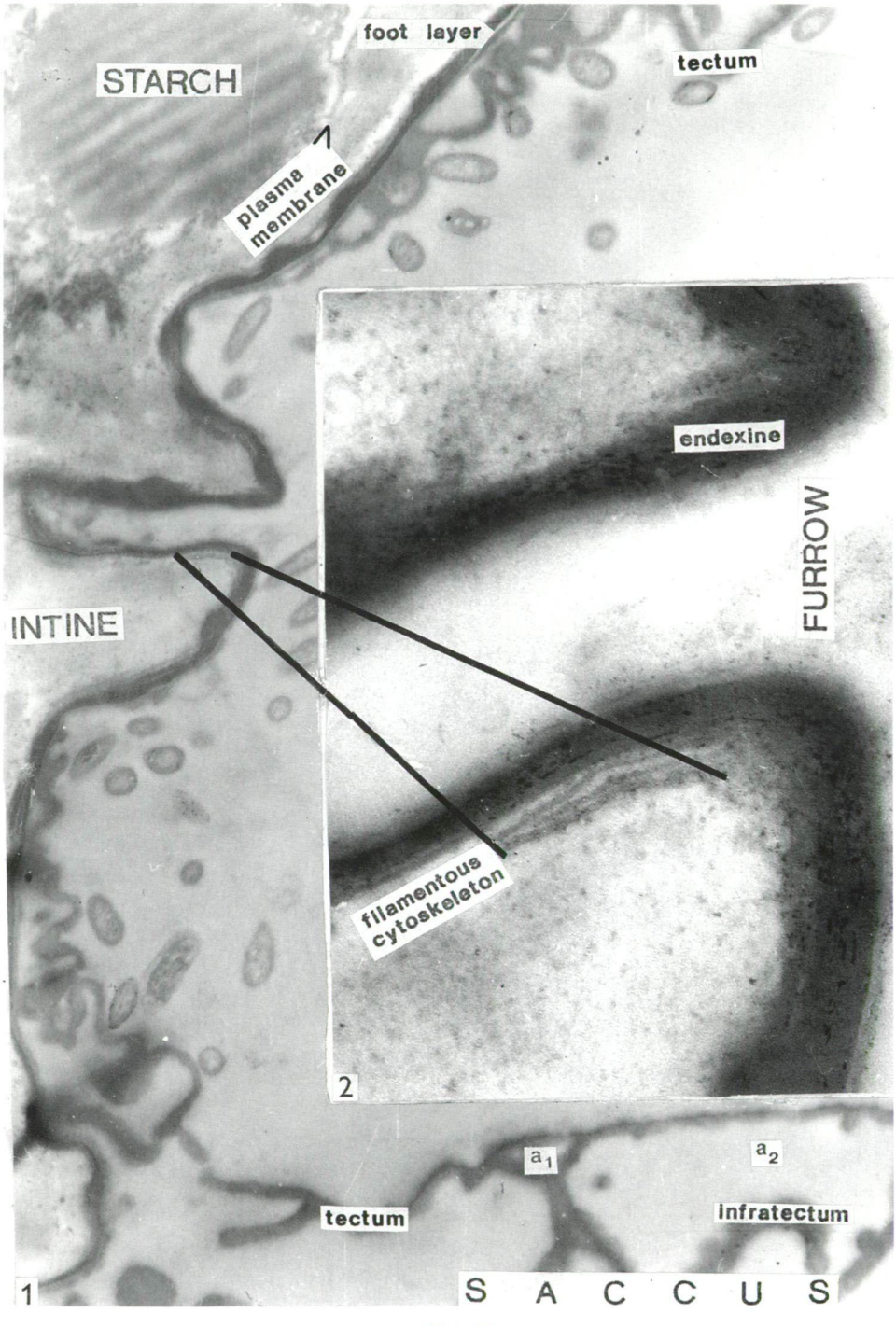
STARCH
foot layer
tectum
plasma membrane
endexine
FURROW
INTINE
filamentous cytoskeleton
2
a1
a2
tectum
infratectum
1
S A C C U S

Plate 7.3.

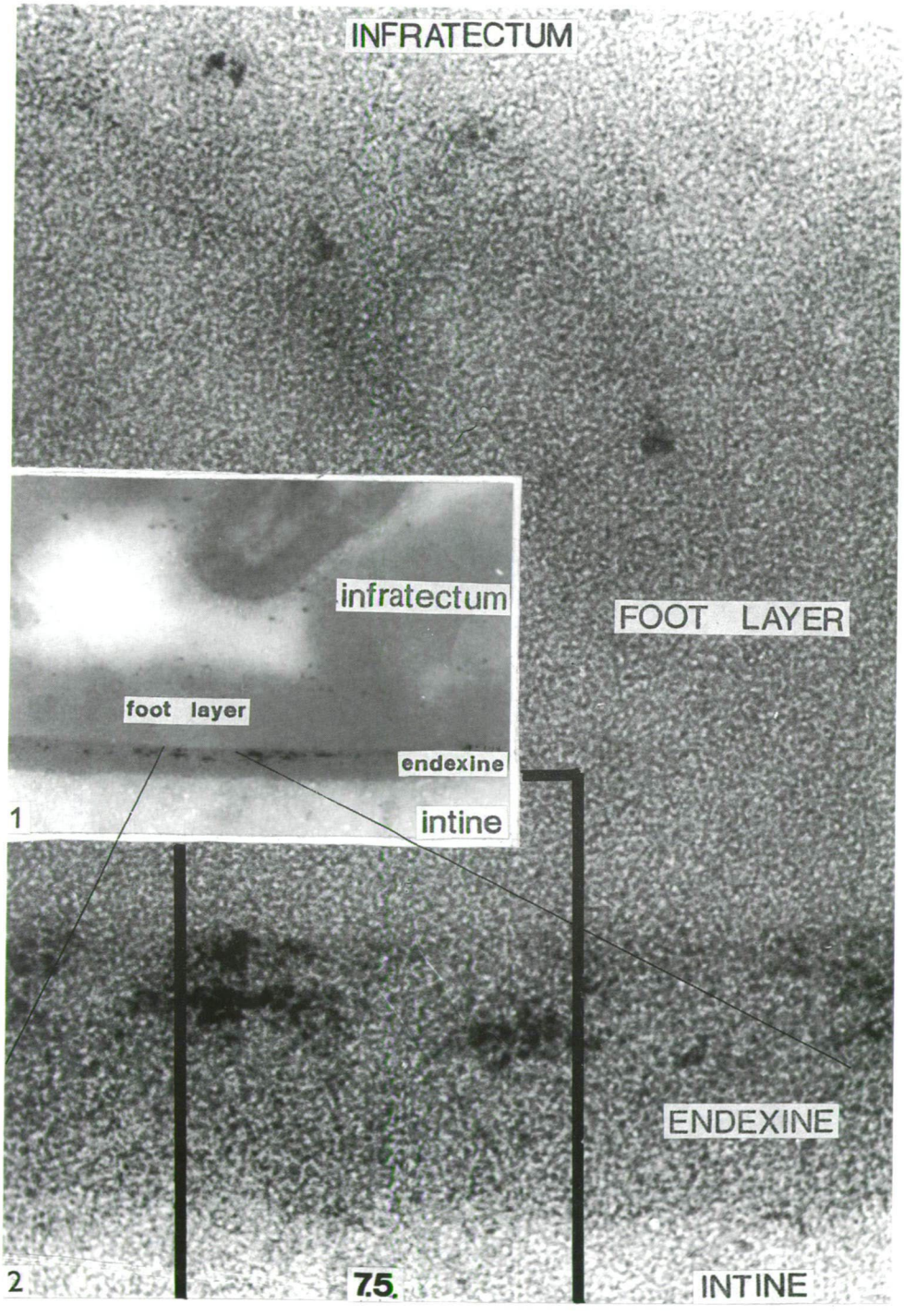
INFRATECTUM
infratectum
FOOT LAYER
foot layer
endexine
1
intine
ENDEXINE
2
7.5.
INTINE

Plate 7.4.

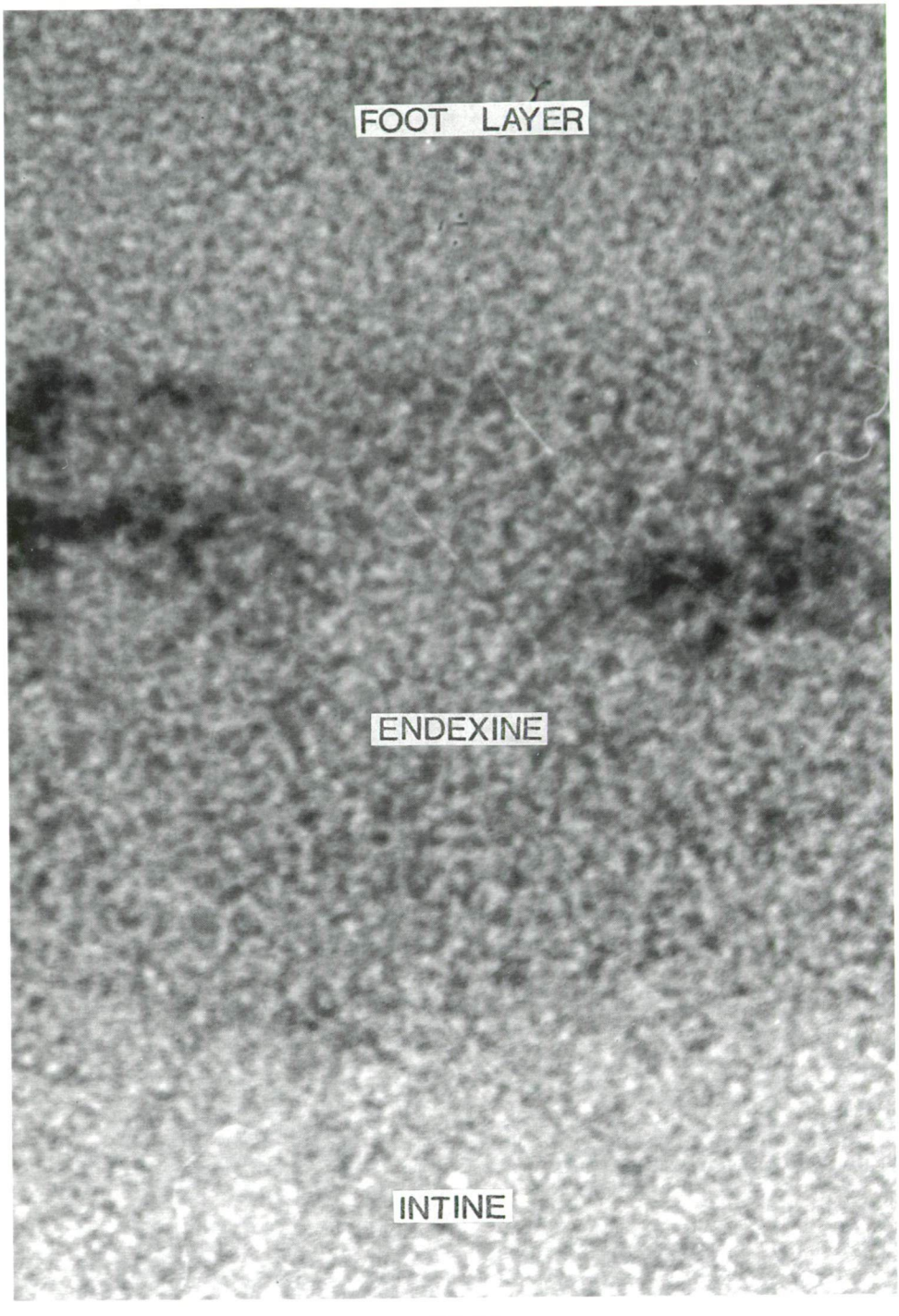

Plate 7.5.

plate 7.5.) of the corpus, remarkable desintegration was observed. Within the endexine there are granular particles with high electron density. The molecular system is very complex (Plate 7.5.) and based on our up-to-date knowledge we can establish the following:

1. The bordering lines of the different layers of the exine (ectexine, endexine, intine) in the highly magnified pictures become continuously less characteristic.
2. A trend toward a homogeneous molecular system may be established. Well shown is in Plate 7.5. that the differences between foot layer and endexine newly disappear. The lamellae are represented by unit-membrane like remnants only. The intine differs from the endexine by its electron density.
3. Chain molecular systems were observed in different orientation. Cyclic molecular structures were also observed in the highly magnified pictures (Plate 7.5.).

Plate 7.1.

1–5. *Pinus griffithii* McCLELL. Recent.
1,2. Experiment No: 1736.
1. Detail of the ectexine ultrastructure of the saccus. Negative no: 6068. 15.000x.
2. Detail of the infratectum. Negative no: 6062. 15.000x.
3–5. Experiment No: 1737.
3. Ultrastructure of the exine of the corpus. Negative no: 6063. 50.000x.
4. Exine ultrastructure of the corpus/saccus border. Negative no: 6065, 6066. 50.000x.
5. Detail of the ectexine ultrastructure of the saccus. Negative no: 6043. 15.000x.

Plate 7.2.

1–4. *Pinus griffithii* McCLELL. Recent. Experiment No: 1738.
1. Exine ultrastructure of the corpus. Negative no: 6059. 15.000x.
2. Detail of the exine ultrastructure of the corpus. Negative no: 6056. 50.000x.
3. Exine ultrastructure of the corpus/saccus border. Negative no: 6057. 15.000x.
4. Detail of the exine ultrastructure of the saccus. Negative no: 6053. 15.000x.

Plate 7.3.

1,2. *Pinus griffithii* McCLELL. Recent. Experiment No: 1739.
1. Ultrastructure in the apertural area. Negative no: 1082. 8.000x.
2. Detail of the ultrastructure in the furrow area. Negative no: 6081. 50.000x.

Plate 7.4.

1,2. *Pinus griffithii* McCLELL. Recent. Experiment No: 1739.
1. Detail of the exine ultrastructure of the corpus. Negative no: 4954. 100.000x.
2. Highly magnified picture of the foot layer, endexine and intine. Negative no: 4955. 1,000.000x.

Plate 7.5.

Pinus griffithii McCLELL. Recent. Experiment No: 1739. Molecular structure of the foot layer, endexine and intine. Negative no: 4955. 2,500.000x.

Discussion and Conclusions

The resistance of the exine layers, and in general of the whole pollen grains to X-ray irradiation of *Pinus griffithii* can be pointed out in the first place. Some organelles of the protoplasm have not altered in a remarkable manner. Filamentous cytoskeleton, the plasma membrane and starch granules have been observed in a more or less well preservation. The molecular system was not so well discovered by the X-ray irradiation as by the organic solvents. But it is worth of mentioning that the results presented in Plate 7.5. are similar to those of *Ustilago maydis* (DE CANDOLLE) CORDA, but after a stronger X-ray dose (300').

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